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A Novel Approach to the Development of Highly Specific Inhibitors of a Critical Transcription Factor in Prostate Cancer

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Using isothermal titration calorimetry (ITC), we have measured the binding affinity of several constructs of ERG to DNA. The results show that there is auto-inhibition of DNA binding by regions outside the conserved Ets domain, as observed for Ets-1. Based on this data, we are focusing our structural efforts on a fragment of ERG which is auto-inhibited. Backbone NMR resonance assignments for this fragment have been completed and sidechain assignments are in progress. Analysis of the backbone chemical shifts shows the expected fold for the Ets domain. Regions outside the Ets domain show some evidence of additional structural elements but will require the complete structure determination for full characterization. We are well positioned now to complete the structure determination of the auto-inhibited form. We have also developed and validated a fluorescence polarization assay for screening effects of compounds which can be used for fragment screening and high throughput screening.

15. SUBJECT TERMS

prostate cancer, ERG, auto-inhibition, DNA binding, transcription factor

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Progress Report

Introduction

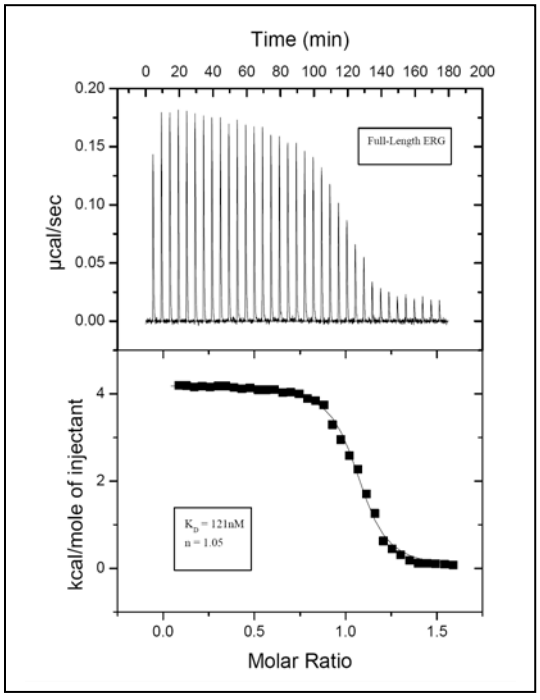
More than 230,000 new cases of prostate cancer were diagnosed in the United States in 2006 and more than 27,000 deaths were attributed to prostate cancer, accounting for ~9% of cancer deaths in men. It is clear that there is a need for more effective highly targeted agents for the treatment of this pervasive cancer. Our goal is to explore a novel approach, namely inhibition of a specific protein, ERG, to treat this deadly disease.

We have proposed three steps to achieve our goal of finding compounds that can inhibit ERG function by stabilizing the inhibited form of the protein. First, we will make ERG in bacteria and identify which parts of the protein mediate the inhibition. Second, we will utilize nuclear magnetic resonance spectroscopy to determine the 3D structure of the inhibited form of ERG. This structural information makes it possible to use computational tools to identify molecules which may bind to ERG. Third, we will use these computational tools to identify potential lead compounds which can stabilize the inhibited form of ERG and inhibit its binding to DNA. Based on the computational results, we will test a selected panel of compounds for their ability to inhibit binding of ERG to DNA to identify compounds which are effective and can be developed further into a useful drug.

Our efforts can lay the foundation for development of drugs targeting ERG which is a novel and potentially therapeutically powerful approach for the treatment of prostate cancer.

Body

Aim 1: Delineation of auto-inhibitory domains of ERG.



Measurement of DNA binding by ERG fragments using isothermal titration calorimetry (ITC). Our previous efforts to measure DNA binding were complicated by some degree of aggregation of the proteins under the conditions we were employing. This has been resolved by employing a higher concentration of KCl, making it possible to get accurate measurements. We have used isothermal titration calorimetry (ITC) to measure the binding of several fragments. Data for full-length ERG are shown in Figure 1. The stoichiometry is very close to 1.0 (N=1.05), indicating that the protein is fully active. Under the conditions employed, the K_d value is 121 nM for an oligonucleotide derived form selection studies with Fli-1 (Mao et al., 1994).

Figure 1. ITC measurement of DNA binding to full-length ERG.

Subsequently, we have measured the Kd for all the fragments we have generated. This data is shown in Figure 2. It is clear that longer forms of the protein are inhibited relative to the conserved Ets domain. The results also indicate that the motifs for auto-inhibition are contained in the aa 251-412 construct, so structural efforts are focused on this construct.

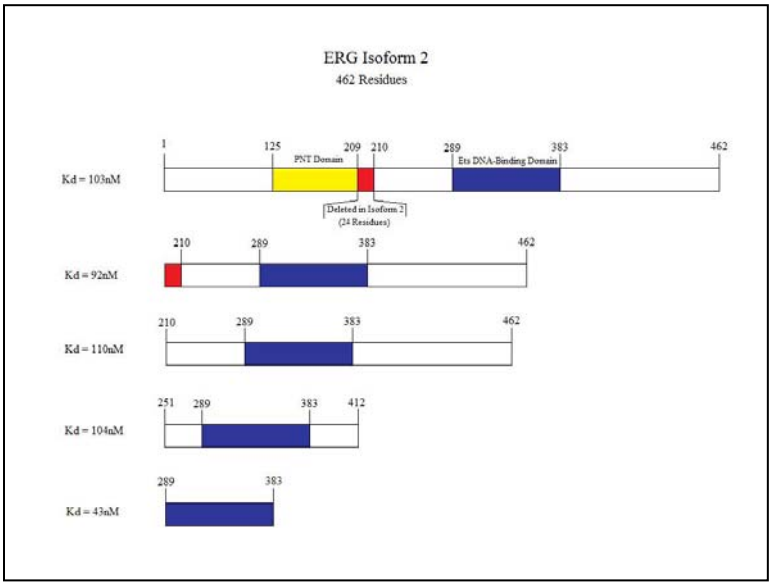


Figure 2. Results of ITC measurements of DNA binding to various fragements of ERG.

Aim 2: Determination of the structure of the auto-inhibited form of ERG (X-ray or NMR).

NMR resonance assignments for ERG. We have labeled the isolated Ets domain (aa 289-383) with ^{15}N and ^{13}C and carried out backbone and sidechain resonance assignments using a panel of heteronuclear triple resonance experiments. Strips from the HNCACB data and from an ^{15}N-edited NOESY are shown in Figure 3. Analysis of the backbone chemical shifts using TALOS for secondary structure shows a high degree of similarity to the structures of known Ets domains.

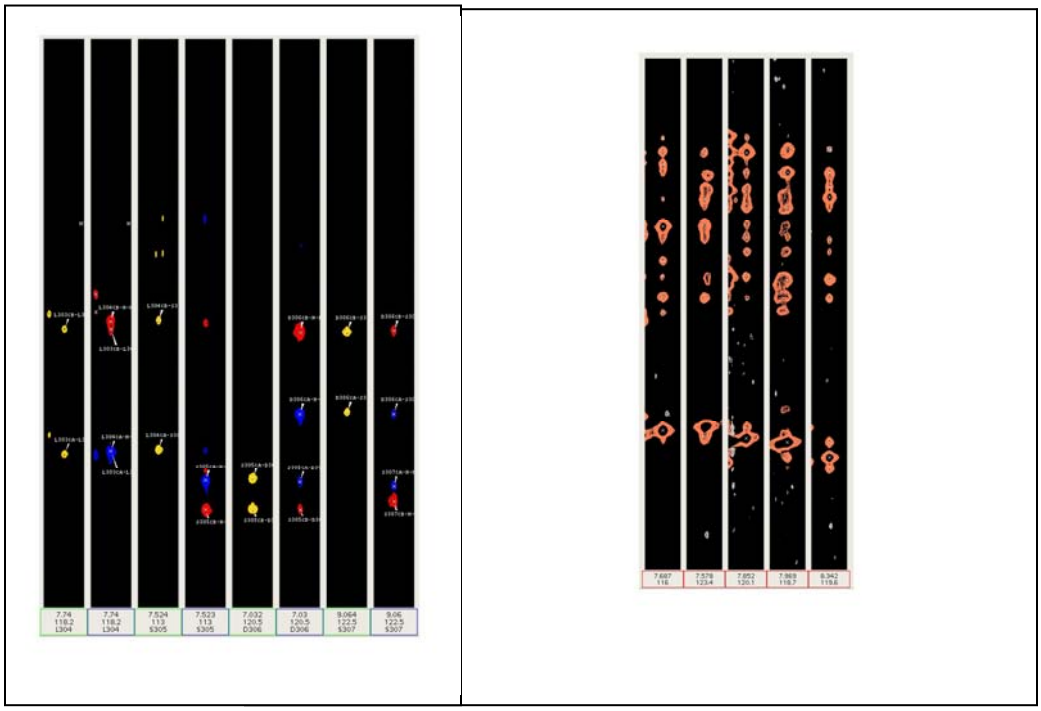


Figure 3. Left Panel: Correlated strips from the 3D HNCACB of ERG(289-383). Right Panel: Selected strips from the 3D ^{15}N-edited NOESY of ERG(289-383).

NMR resonance assignments for ERG(251-412), the auto-inhibited form. We have labeled ERG(251-412) with 13C and 15N and recorded a series of triple resonance experiments for backbone and sidechain resonance assignment. Backbone resonance assignments have been completed and sidechain assignments are underway. Figure 4 shows an overlay a selected region of the 15N-1H HSQC spectra of ERG(289-383), the Ets domain, and ERG(251-412), Ets domain plus auto-inhibitory regions. Interestingly, residues in the core Ets domain show chemical shift changes in the longer form of the protein. This is clear evidence that regions outside the Ets domain, the auto-inhibitory elements, make contact with the core domain. This provides strong justification for our effort to solve the structure of the auto-inhibited form of the protein.

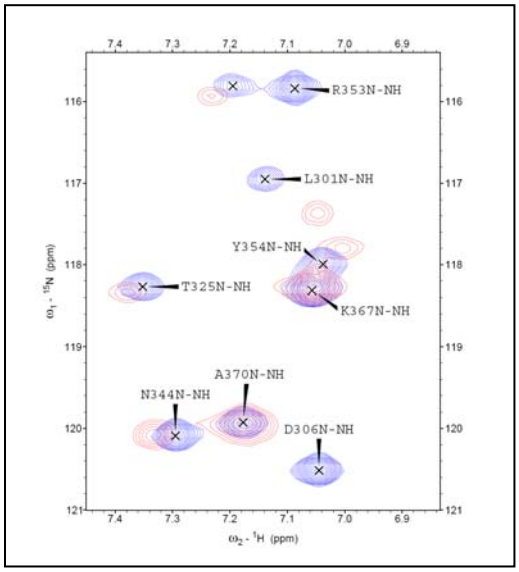


Figure 4. Overlay of ^{15}N-^{1}H HSQC spectra of ERG(289-383) in blue and ERG(251-412) in red.

Aim 3: Virtual screening of ERG structure to identify potential lead compounds.

We do not yet have a structure of the protein, so efforts on this aim have not yet begun.

Key Research Accomplishments

- Measurement of binding of a series of ERG fragments by ITC
- Demonstration of auto-inhibition for ERG
- NMR resonance assignments for auto-inhibited ERG
- NMR evidence for interaction of auto-inhibitory elements with the Ets domain of ERG

Reportable Outcomes

- We plan to use the preliminary data generated under the auspices of this grant to seek additional federal funding for this project through DOD or NIH.
- One student, Michal Walczak, has been supported by this grant.

Conclusion

We have identified an auto-inhibited fragment of ERG which will now serve as the focus of our structural efforts. This work lays the foundation for initiating efforts to develop small molecule inhibitors of this protein.

References

Mao, X., Miesfeldt, S., Yang, H., Leiden, J. M., and Thompson, C. B. (1994). The FLI-1 and chimeric EWS-FLI-1 oncoproteins display similar DNA binding specificities. J Biol Chem *269*, 18216-18222.